After a day, it was poured into ice water and the mixture was stirred. The reaction product was extracted with chloroform, and the solution was washed with water, dried, and evaporated. After recrystallization from ethanol, the acetyl derivative of (I) with mp 197-198°C (II) was obtained.

PMR spectrum (in $CDCl_3$): 1.96-2.03, 12H (four aliphatic CH_3COO groups at C-2",3",4",6"), 2.24 (s, aromatic CH_3COO group at C-4'), 2.28, 6 H (s, CH_3COO groups at C-3, 8); 2.36 (s, CH_3COO– at C-5); 3.65 and 3.90 (dd, $J_{4",5"}$ = 6.5 Hz, H-5"); 3.80 (s, OCH_3); 4.05-4.25 (m, 2H-6"), 4.95-5.29, 4 H (m, H-1",2",3",4"); 6.73 (s, H-6), 7.04 (d, 8.5 Hz, H-5'); 7.21 (d, 2.5 Hz, H-2'); 7.25 (q, 2.5 and 8.5 Hz, H-6').

SUMMARY

The presence of flavonoids in *Haplophyllum perforatum* has been revealed. From the epigeal part of this plant a new acylated flavonoid glycoside haploside A has been isolated, and its structure has been determined as 3,4',5,7,8-pentahydroxy-3'-methoxyflavone 7-O-(6"-O-acetyl-β-D-glucopyranoside).

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FLAVONOID GLYCOSIDES OF SPORE-BEARING STEMS OF *Equisetum arvense*

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In an extract of spore-bearing stems of *Equisetum arvense* L. (field horsetail) saponaretin, apigenin 5-glucoside, luteolin 5-glucoside, kaempferol 3-sophoroside, quercetin 3-glucoside, 4-hydroxy-6-(2-hydroxyethyl)-2,2,5,7-tetramethylindanone, and a compound of ketonic nature, isolated previously from an extract of the herbage of the field horsetail, have been identified by high-performance liquid chromatography. The characteristics of the chromatographic behavior of glycosylated flavones under the conditions of reversed-phase liquid chromatography have been studied and it has been shown that the glycosylation of flavones at position 5 of the molecule causes a greater fall in the affinity for a nonpolar stationary phase than in position 7 of the molecule.

Phenolic acids and flavonoid aglycones have been identified in the form of their TMS ethers previously in an extract from spore-bearing stems of *Equisetum arvense* (field horsetail) by the gas–liquid chromatography (GLC) method [1]. Using high-performance liquid chromatography, we have now identified flavonoid glycosides in this extract without their

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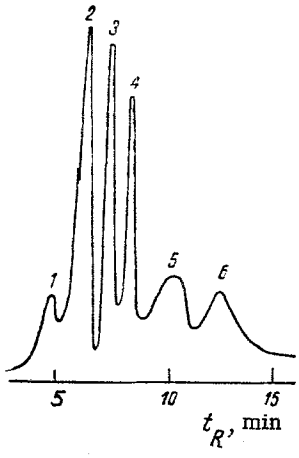

Fig. 1. Chromatographic separation of some flavone and flavanone glycosides: 1) naringein 5-glucoside; 2) naringenin 7-glucoside; 3) luteolin 5-glycoside; 4) luteolin 7-glucoside; 5) apigenin 5-glucoside; 6) apigenin 7-glucoside.

previous conversion into derivatives. We used compounds isolated from the herbage of the field horsetail [2-5] as standards.

Reversed-phase liquid chromatography is sensitive to fine differences on the structures of flavonoid aglycones and their glycosides [6-8]. In the case of flavonol glycosides it has been shown that the introduction of a carbohydrate residue into the flavonol molecule considerably reduces its affinity for a nonpolar stationary phase (SP). The position of the carbohydrate residue is important here [8].

The flavnoid glycosides studied previously contained the carbohydrate residue either in position 3 or in position 7 [8]. In view of the finding in the field horsetail of 5-glucosides of apigenin and of luteolin, which are rarely found in nature, it was considered of interest to study the chromatographic behaviors of these compounds under the conditions of high-performance liquid chromatography in comparison with their analogs substituted in position 7 (see below). It is known that flavones and flavonols glycosylated in position 5 or 7 have close or identical R_f values in different systems in traditional chromatography, which makes their separation and identification difficult [9, 10].

The results that we have obtained have shown that under the conditions of reversed-phase liquid chromatography separation of apigenin, luteolin, and naringenin 5- and 7-glucosides takes place fairly clearly (Fig. 1).

Below we give the retention times (t_R, min) of flavone and flavanone glycosides [eluent: water–methanol–acetic acid (90:5:5 by volume)]:

Aglycone		7-Glucoside	5-Glucoside
Naringenin	35.00	6.20	5.00
Luteolin	23.00	8.60	7.00
Apigenin	30.00	12.00	10.60

As we can see, the glycosylation of flavones and flavanones sharply lowers their affinity for a nonpolar phase, as in the case of flavonols [8]. At the same time, glycosylation in position 5 causes a greater reduction in t_R than in position 7. Hydroxy groups differ in affinity according to their positions in the flavonoid molecule. While a 7-OH group possesses a high affinity [11], a 5-OH group has a lower value of the ionization constant, since it is bound by an intramolecular hydrogen bond (intra-HB) to the oxygen atom of the carbonyl group [12].

Since on the glycosylation of a 7- or a 5-OH group by a glucose residue the same number of aliphatic hydroxy groups is introduced into the flavone molecule, the appearance of similar chromatographic properties might be expected and this has in fact been found under the conditions of classical methods of chromatography. However, when a 5-OH group is glycosylated the intra-HB is broken and, by analogy with 5-methoxyflavone [13], one may expect an increase in the basicity of the compound through the liberation of the carbonyl group. This leads to a change in the polarity of the molecule, which is well shown under the conditions of reversed-phase chromatography and makes it possible to separate these compounds with a fairly high efficiency.

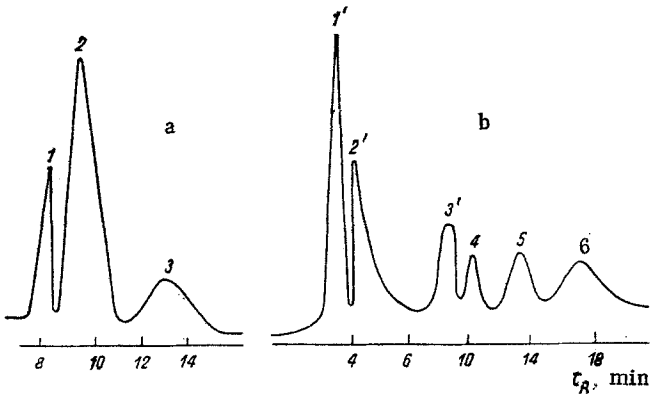

Fig. 2. Chromatographic separation of the compounds of individual fractions from an extract of spore-bearing field horsetail stems [a) eluent A; b) eluent B]: 1) kaempferol 3-sophoroside; 2) apigenin 6-C-glucoside; 3) compound A; 1') kaempferol 3-sophoroside; 2') apigenin 6-C-glucoside; 3') quercetin 3-glucoside; 4) luteolin 5-glucoside; 5) apigenin 5-glucoside; 6) 4-hydroxy-6-(2-hydroxyethyl)-2,2,5,7-tetramethylindanone.

Using high-performance liquid chromatography we have separated and determined the composition of a number of fractions from an extract of spore-bearing field horsetail stems [1]. In addition to flavonoid glycosides, we have established the presence in them of 4-hydroxy-6-(2-hydroxyethyl)-2,2,5,7-tetramethylindanone [14] and of a compound of ketonic nature (compound A) the structure of which is being determined at the present time.

The retention times (t_R, min) of the components present in the fractions of the extract of spore-bearing field horsetail stems are as follows (eluent: water–methanol the following ratios by volume: A, 90:10; B, 80:20):

Component	A	B
Kaempferol 3-sophoroside	8.00	3.90
Apigenin 6-C-β-D-glucopyranoside (saponaretin)	9.40	4.40
Quercetin 3-O-β-D-glucopyranoside	—	9.00
Luteolin 5-O-β-D-glucopyranoside	—	10.00
Apigenin 5-O-β-D-glucopyranoside	—	13.80
Compound A	14.20	—
4-Hydroxy-6-(2-hydroxyethyl)-2,2,5,7-tetramethylindanone	—	17.00

In finding conditions for the more complete separation of the fractions we widely varied the composition of the eluent systems with a change in the proportion of methanol from 5 to 50 vol.%, both with the addition of acetic acid (2 vol.%) and without it. This enabled us to check the presence of aglycones in the fractions. In actual fact, in the more polar mobile phases containing between 30 and 50 vol.% of methanol chromatographic peaks appeared that corresponded to luteolin and apigenin. Thus, it has been shown that in the fractions enriched with flavonoid glycosides that were analyzed there were also very small amounts of certain aglycones (luteolin, apigenin). However, for analyzing the flavonoid glycosides it proved to be more rational to use the eluent systems A and B as mobile phases. In system A there is a better separation of the more polar compounds (Fig. 2a) and in system B a fairly effective separation of fractions containing flavonoid glycosides and 4-hydroxy-6-(2-hydroxyethyl)-2,2,5,7-tetramethylindanone (Fig. 2b).

On the whole, it can be stated that the spore-bearing stems of the field horsetail contain all the flavonoid glycosides and also the 4-hydroxy-6-(2-hydroxyethyl)-2,2,5,7-tetramethylindanone and compound A that are characteristic for the herbage of the field horsetail.

EXPERIMENTAL

Analysis was carried out on a Perkin-Elmer 1220 liquid chromatograph with a UV detector at a wavelength of 254 nm. Column 50 cm × 5 mm (internal diameter) with the sorbent ODS-Sil-X-II (35 ± 15 μm). The eluents consisted of mixtures of water and methanol and of water, methanol, and acetic acid in various proportions (see above). It was found that an increase in the proportion of methanol or acetic acid led to a decrease in the retention times of the components.

An individual compound or a sample of the initial fraction (1 mg) was dissolved in 1 ml of the appropriate eluent system, and 0.5 to 2 μl of the solution was deposited on the column. The rate of elution was 1 ml/min, and the sensitivity 0.050 OD.

SUMMARY

1. The following compounds have been identified in an extract of spore-bearing stems of the field horsetail by high-performance liquid chromatography: saponaretin, apigenin 5-glucoside, luteolin 5-glucoside, kaempferol 3-sophoroside, quercetin 3-glucoside, 4-hydroxy-6-(2-hydroxyethyl)-2,2,5,7-tetramethylindanone, and compound A.

Features of the chromatographic behavior of glycosylated flavones under the conditions of reversed-phase liquid chromatography have been studied and it has been shown that glycosylation in position 5 causes a greater fall in affinity for a nonpolar mobile phase than glycosylation in position 7.

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